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CONTENTS

ABBREVIATIONS

BMI	body mass index
Body fat%	body fat percent
CNS	central nervous system
DXA	dual-energy X-ray absorptiometry
FFM	fat free mass
FM	fat mass
FSH	follicle-stimulating hormone
GH	growth hormone
HR	heart rate
IGF-1	insulin-like growth factor 1
IL-6	interleukin-6
LDL	low density lipoprotein
LH	luteinizing hormone
NPY	neuropeptide Y
RPM	repetition per minute
SD	standard deviation
VO_{2peak}	peak oxygen consumption
$VO_{2peak/kg}$	peak oxygen consumption per kilogram body mass

LIST OF ORIGINAL PUBLICATIONS

I. Cicchella A., Jürimäe T., Stefanelli C., Purge P., Lätt E., Saar M. Correlation of skinfold thicknesses and circumferences at exactly defined body sites with leptin in 10–12-year-old boys with different BMI. Collegium Antropologicum (In Press).

II. Cicchella A., Stefanelli C., Purge P., Lätt E., Saar M., Jürimäe T. The association between peak O_2 consumption and leptin in 10- to 12-year-old boys. Clinical Physiology and Functional Imaging 2013, 33(4): 313–316.

III. Cicchella A., Stefanelli C., Jürimäe T., Saar M., Purge P. Moderate physical activity correlates with elevated leptin in normal BMI and physically active 10–12-year-old boys. Perceptual and Motor Skills 2013, 117 (2): 1–9.

The dissertant had primary responsibilities for protocol development, subjects' screening, performing measurements, data analysis and writing all the articles.

I. INTRODUCTION

The regulation of body mass is a topic of major concern for health worldwide and the number of obese children and adolescents has increased in the past ten years (Ogden et al. 2012). Obesity can often begin in childhood, and the transition between childhood and adolescence (peripubertal phase) is a critical stage for the physical development of healthy children (Martos-Moreno et al. 2013). Leptin has been shown to be the key hormone involved in fat metabolism in children and to some extent predict body fat accumulation (Kettaneh et al. 2007).

Different studies have been published about the correlations between fat metabolism, physical activity, exercise capacities and habits and body composition in peripubertal boys (Clayton et al. 1997, Roemmich et al. 1998, Horlick et al. 2000, Shalitin and Phillip 2003, Kettaneh et al. 2007, Polotsky et al. 2012, Sahin-Efe et al. 2012). It is known that the adipocytokines, and especially leptin hormone, have a key role in linking development, fat metabolism (as a rule increasing in direct proportion with increasing adiposity) and exercise capacities in peripubertal children (Blum et al. 1997, Jéquier and Tappy 1999, Kramer et al. 2002, Hosick et al. 2010, Fugua and Rogol 2013). Leptin levels serve as an adiposity sensor to protect against starvation and correlate with the degree of obesity; thus leptin probably has a permissive role in high energy metabolic processes occurring during puberty (Weiss et al. 2013).

The existing studies do not specifically consider full anthropometry (using only some skinfold thicknesses) (Roemmich et al. 1998), or peak aerobic capacity (Andreacci et al. 2005). Tanner stage maturation (sexual development) and leptin studies in boys have not been stratified for normal, overweight and obese children (Blum et al. 1997) and do not consider skinfolds, circumferences and lengths, but only FM and FFM, without considering the regional distribution of FM in the body. Adipose tissue distribution is a strong predictor of the occurrence of the metabolic syndrome in the context of obesity (Wang et al. 2013).

Behavioral studies linking levels of physical activity and leptin are also relatively few (Barbeau et al. 2003, Romon et al. 2004, Metcalf et al. 2009, Jimenez-Pavon et al. 2012, Martinez-Gomez et al. 2012). Previous studies indicate that for decreasing body fat mass and leptin, moderate-to-vigorous physical activity is needed as a rule. There is no information on large samples of peripubertal boys in the same Tanner stage and of different body (fat) mass about the relationships between leptin levels, anthropometry, VO_{2peak}, and behavioral habits (levels of physical activity).

As the regulation of fat mass is dependent on the ratio of energy intake and energy expenditure, a signaling mechanism is necessary for regulating this balance. Leptin hormone, acting as a sensor that monitors the size of adipose tissue mass through specific leptin receptors in hypothalamic centers is the link factor between energy expenditure and energy intake (Kraemer et al. 2002). There is also evidence that leptin may be the link between body fat and earlier

puberty (Jéquier and Tappy 1999). Leptin-deficient mice and humans fail to enter puberty unless leptin is administered, and recent studies indicate that very low levels of leptin stimulate gonadotropin secretion both at the hypothalamic and the pituitary level (Garcia-Mayor et al. 1997, Kaplowitz 2008). Many factors are acting simultaneously, regulating the mechanism of puberty onset, and it remains unclear how these factors interact with each other. Furthermore, the differences in the relationship between puberty onset and leptin levels in peripubertal boys with normal BMI, boys who are overweight and boys who are obese are contradictory. The main task of this dissertation is to study possible relationships between leptin, skinfold thicknesses and circumferences, peak O_2 consumption and different levels of physical activity (obligatory physical education classes at school, participation in sport sections, and free-time physical activity) in peripubertal boys with different BMI.

2. REVIEW OF THE LITERATURE

2.1 Discovery of Leptin and Relationship with Other Biomarkers

Adipocytokines, hormones produced mostly by white fat cells, have been studied as regulators of fat metabolism and appetite. It has been found that adipocytokines play a fundamental role in obesity development (Kiess et al. 2001, Lehr et al. 2012). Among adipocytokines, leptin emerges as the most promising marker of detecting the possibility of developing obesity in children (Cheng et al. 2008). Leptin is also a major regulator of energy uptake and homeostasis in the organism (Haalas et al. 1995). Other hormones of the adipocytokines family, produced by fat tissue, seem to be important as signaling mediators of appetite (Devos et al. 1996, Kiess et al. 2001). The connection of different adipocytokines with physical activity has been extensively studied (Venner et al. 2006, Jürimäe et al. 2007, Morris 2008).

In 1950, animal caretakers at Jackson Laboratories (USA) discovered the ob/ob mouse (Ingalls et al. 1950) who showed recessive genetic obesity resulting in sterile adult mice with over 50% body fat. Due to the gene mutation, the ob/ob mouse did not produce leptin. Coleman (1973) reported similar results in studies using parabiosis (cross-circulation) of these mice. Their research showed that when crossing an ob/ob mice with a db/db mice (also obese but hyperglycemic), a decrease of food intake and body mass occurred in the ob/ob mice and an increase in body mass in the db/db mice. Coleman (1973) concluded that ob/ob mice fail to produce a circulating factor from adipose tissue but their brain can respond to it and reduce food intake, whereas db/db mice produce the circulating factor in their adipose tissue but their brain cannot respond to it. Friedman (1995) demonstrated the gene defect in the ob/ob mice. This discovery led to the publication of many papers on leptin, fostered by substantial investments of the industry aiming at the possible application of leptin in fighting obesity. The advent of biotechnological tools made it possible to verify Friedman's hypotheses. Three papers in 1995 clearly demonstrated that the ob protein, leptin, eliminated the obesity in the ob/ob mice (Halaas et al. 1995, Pelleymounter et al. 1995, Rentsch et al. 1995). It has to be noted that this mutation is very rare in man and only 14 cases and 4 pathogenic mutations worldwide have been described (Fatima et al. 2011).

Leptin (derived from the word *leptos* in Greek, meaning "lean") was discovered by Zhang and coworkers in 1994 (Zhang et al. 1994) and is an adipocyte derived 16–kDa hormone, produced by white adipose cells (Considine et al. 1996). In children and adolescents, the number of adipocytes in white adipose tissue has been proven to increase with growth (Spalding et al. 2008). Leptin resistance or deficiency in humans results in obesity, diabetes and infertility. Leptin is encoded by the ob genes both in humans and mice (Haalas et al. 1995). It circulates in serum both as free and protein bond, mainly bond to

the soluble isoform of its receptor, and its level in the blood stream changes little during the day, reaching the peak at night (Meier and Gressner 2004).

Structural studies have demonstrated that leptin is a member of the four-helical cytokines subfamily, together with the granulocyte-colony stimulating factor, growth hormone, erythropoietin, interleukins and leukemia inhibitory factor (Zhang et al. 2005). In this structural family, leptin resides close to IL-6 and other important functions of leptin have been discovered, such as its role in angiogenesis, hematopoiesis, blood pressure regulation, bone mass turnover, lymphoid organs homeostasis and T-lymphocyte production (Houseknecht et al. 1998).

Leptin expression is greater in subcutaneous than in visceral adipose tissue, and females have normally higher levels of leptin since they have more subcutaneous fat than males (Gueorguiev et al. 2001). Pubertal females, after correction for fat content, show the threefold content in leptin in comparison to male pubertal subjects. This effect is thought to be caused by androgen hormones that reduce the circulating serum leptin in males (Roemmich et al. 1998).

Growth hormone and cortisol have been demonstrated to increase leptin production, while catecholamines depress leptin production (Kramer et al. 2002) and exogen leptin increases GH levels (Tannenbaum et al.1998). Despite the first promising results got in treating obesity in rats, leptin is no longer perceived as a single cause in humans that acts on the regulation of various organs and contributes to obesity but rather as a signaling hormone that works with other mechanisms. In their review article, Gat-Yablonski and Phillip (2008) concluded that leptin stimulates linear growth (e.g. of bone epiphysis) of the body by regulating the energy balance and by stimulating the secretion of GH that is simultaneously involved in bone remodeling and has a direct effect on the condrocytes of the growth plate.

Leptin was proven to correlate positively with the relative change in fasting insulin, the relative change in LDL cholesterol at 2 months, percentage fat loss (Kirel et al. 1999) and change in BMI at 2 and 6 months in obese children participating in a weight-loss inpatient program (Murer et al. 2011). Leptin was also proven to correlate positively with the change in BMI in obese humans (Misra et al. 2001). Celi et al. (2003) found that in obese boys the relapse after weight loss was connected with leptin levels. It is proven that human obesity is characterized by excessive leptin production and by varying degrees of leptin resistance or deficiency. In a study examining a total of 25 obese children of the Punjabi region of Pakistan (Fatima et al. 2011), leptin deficiency was detected in nine. In these subjects, leptin gene sequence identified mutations in homozygous state in all leptin- deficient children. Leptin resistance seems thus to reside in the transport system. When leptin is administered centrally in rats, its action persists, whereas when it is administered peripherally, its action decreases gradually.

Leptin can also be found in the same areas of the brain where the NPY is produced. Hypothalamus NPY receptors (Y1, Y2, Y4 and Y5) have been found in various limbic structures (Kohno and Jada 2012). The limbic system is

involved in the emotional status regulation, this suggests a possible role for leptin in linking nutrition habits and emotional state. In the arcuate nucleus of the brain, ghrelin mainly activates NPY neurons. Leptin and insulin suppress the ghrelin-induced activation in 30–40% of the ghrelin-activated NPY neurons (Kohno and Jada 2012). NPY in arcuate neurons serves as a downstream mediator of leptin in maintaining energy homeostasis (Bi et al. 2012).

Relationship between leptin, BMI and the sum of skinfolds was found in normal-weight children and adults (Kettaneh et al. 2007). Roemmich et al. (1998) found in prepubertal and pubertal children and adolescents a relationship between the sum of 9 skinfolds and circulating leptin levels, but failed to establish associations with specific skinfolds.

Leptin levels seem to be affected by exercise, and especially by long-term aerobic exercise (Kramer et al. 2002). A recent large study (the Helena Study, Jimenez-Pavon et al. 2012) measured physical activity, body composition and leptin concentration in the group of 902 adolescent boys and girls from eight European countries. The results indicate that physical activity, specifically vigorous physical activity, is negatively associated with leptin concentrations after controlling for potential confounders including total body fat (Jimenez-Pavon et al. 2012). These results are in agreement with some previous studies (Platat et al. 2006),but others failed to find an association between objectively assessed physical activity and leptin (Metcalf et al. 2009). Platat et al. (2006) showed that total physical activity assessed by questionnaire was inversely associated with leptin after controlling for body fat in adolescents. Similarly, Romon et al. (2004) found a negative association of physical activity (assessed by both questionnaire and pedometer) with leptin in girls, also after controlling for body fat. The same result (leptin negatively correlated with physical activity in children and adolescents <18 years) was found in a study considering 414 (209 boys and 205 girls) children and adolescents (Kettaneh et al. 2007).

The correlation of leptin and body fat depots, skinfold thicknesses and circumferences, aerobic capacity and physical activity levels in children of different body mass is still unknown.

2.2 Leptin and Puberty Onset in Boys

Puberty involves the activation and maturation of the hypothalamic-pituitary-gonadal axis and during its course rapid changes occur in body size and shape (Grunbach and Styne 2011, Lazar and Philip 2012). Age at the onset of puberty differs in healthy boys from 11 to 12 years, ranging by 2–3 years (Lazar and Philip 2012). The chronological age does not necessarily reflect the pubertal maturity status. Body fat is an important element of pubertal growth (Maor et al. 2002). Boys who had greater fat mass (sum of skinfolds) had a less advanced sexual maturation stage by the age of 12 years (Biro et al. 2006). A positive relationship was found between age at the onset of puberty and BMI at pubertal onset (Vizmanos and Marti-Henneberg 2000). Boys develop musculature, their

fat mass is decreased and they undergo pubertal growth spurt (Viswanathan et al. 2012). Changes occurring in the pubertal stage are connected with the changes in body fat distribution. The latter are probably dependent on the location of fat tissue mass on the body – e.g. the lipolytic activity of the abdominal region is higher than other parts of the body. Circulating leptin concentration is associated with total body fat mass. However, it is unknown whether there are any differences in this relationship between leptin and fat distribution during puberty (Viswanathan et al. 2012).

Leptin plays a crucial role in pubertal growth (Maor et al. 2002), activating the leptin receptors in the growth plate, stimulating IGF-1 receptor gene expression and abundance in the growth center, independent of the prevalence of GH (Maor et al. 2002).

Leptin has a specific role in stimulating the activity of enzymes essential for the synthesis of adrenal androgens (Shalitin and Phillip 2003). At relatively high levels, leptin can facilitate the onset of sexual maturation, while at low levels it can inhibit this process. In the transition phase between childhood and adulthood the initially overweight subjects have an earlier sexual development compared to normal-weight subjects, while obese children showed a retarded onset of puberty (Shalitin and Phillip 2003, Michalakis et al. 2012). In general, leptin may serve as the signal from fat depot to the brain about the adequacy of fat stores necessary for pubertal development (Kiess et al. 2001). Finally, several studies have discussed extensive interindividual variations in the onset of normal puberty (Marshall and Tanner 1970, Slyper 2006, Tomova et al. 2010).

Relationships between leptin and Tanner stages have been studied previously (Blum et al. 1997, Roemmich et al. 1998, Horlick et al. 2000, Jeffery et al. 2012). Leptin level has been shown to reach the highest level in boys by Tanner stage two (Roemmich et al. 1998, Casazza et al. 2010). This supports the notion of a stimulating effect of testosterone on leptin levels in boys (Brandao et al. 2003). At Tanner stage two, leptin correlated strongly with the sum of skinfolds and percent body fat ($r=0.6–0.8$) in both sexes, and regression analysis showed the changes in leptin level over the 3 years preceding the onset of puberty were not associated with age at the onset of puberty in boys (Jeffery et al. 2012). Besides, there are differences in the relationship between leptin and Tanner stages in males and females, females having high leptin concentration at Tanner stage four, while in males leptin is inversely correlated with testosterone concentration (Blum et al. 1997, Horlick et al. 2000). Gonadal steroids thus seem a potent determinant of leptin levels. Garcia-Mayor et al. (1997) found leptin to be the first hormone to increase together with GH in males during growth, except for leptin dropping after the 10^{th} year while GH is increased. Leptin probably plays a permissive role in the onset of puberty, instead of being its main determinant (Gueorguiev et al. 2001). Finally, Gill et al. (1999) concluded that leptin concentrations expected for a given age and BMI can be associated with constitutional delay in puberty. It is important to understand how this hormone varies at the onset of adolescence in order to prevent obesity that can be associated with pubertal onset.

2.3 Dependence of Anthropometric Parameters on Leptin

BMI is frequently used as a general parameter of fatness in boys (Cole et al. 2000). BMI reference points have advantages over BMI centiles (Jebb and Prentice, 2001). However, BMI does not differentiate between muscle and fat. On the other hand, Daniels (2009) describes BMI as the most useful method for assessing adiposity in the clinical setting in youth, althought Mantzoros et al. (2011) emphasize the high interindividual variability of leptin for a given BMI.
Leptin is produced mostly by white cells adipocytes and is positively related with FM and negatively related with FFM content in the human body (Venner et al. 2006). Some studies have indicated that leptin is produced more by subcutaneous fat tissue than from omental fat (Van Harmelen et al. 2002, Wronska and Kmiec 2012). This is probably the reason why leptin was found to be correlated with the sum of skinfolds in different studies (Ellis and Nicolson 1997, Roemmich et al. 1998, Kettaneh et al. 2007, Swanepoel et al. 2007, Liddle et al. 2012). In all these studies the number of measured skinfolds and circumferences was limited. Kettaneh et al. (2007) measured only the abdominal circumference, Swanepoel et al. (2007) measured six skinfolds but not any circumferences. Liddle and Nicolson (2011) measured only the triceps skinfold. Ellis and Nicolson (1997) calculated only FM and FFM, while Roemmich et al. (1998) measured nine skinfolds but not any circumferences. Pubertal fat distribution is based on the continuous interaction of GH, sex steroids, cortisol, insulin and a variety of other factors (Viswanathan et al. 2012).

The relationship between leptin levels and subcutaneous fat depots is unclear and it is necessary to assess whether there is a dependence of some specific fat depot (skinfold) on leptin levels. Kettaneh et al. (2007) found in adults that a high baseline level of leptin was related to the sum of subcutaneous fat mass (four skinfolds: biceps, triceps, suprailiac and subscapular) while they did not establish a similar relationship in children. In this study (Kettaneh et al. 2007) the baseline leptin predicted an increase in the hip circumference in children at one year follow-up. Another study that measured skinfolds, circumferences and diameters in children showed a relationship between leptin and total subcutaneous fat depots (Roemmich et al. 1998), but did not provide a hierarchy of correlations between each skinfold and leptin levels. After logarithmic transformation this study found leptin to be a predictor of the sum of nine skinfolds (subscapular, chest, mid-axillary, suprailiac, abdominal, triceps, biceps, thigh, and medial calf) in a group of prepubertal and pubertal boys and girls. To our knowledge, there is only one study where the triceps skinfold is used at an earlier age to predict later obesity, but only 5-year-old children were researched (Liddle et al. 2012). Relationships found between anthropometry and leptin have included mainly the sum of skinfolds and few correlations have been found with body fat % (Swanepoel et al. 2007), albeit the ratio of leptin/FM remained invariant across Tanner stages (Ellis and Nicolson 1997, Roemmich et al. 1998). In conclusion, leptin levels have been found to be correlated from the anthropometrical parameters with the sum of measured

skinfolds. The existing studies are limited in sample size, or in the number of anthropometric parameters measured (especially circumferences). Besides, the subjects have not been distributed to lean, normal and obese.

2.4 Relationship Between Leptin and Aerobic Capacity

Some studies have concluded that obesity is often associated with reduced exercise tolerance in children (Drincard et al. 2001). Goran et al. (2000) found that total fat does not influence maximal aerobic capacity, whereas Potter et al. (2013) recently demonstrated that overweight children exhibit slower VO_2 kinetics responses compared to control group children during moderate-intensity cycling. Finally, Gutin (2011) and Rodrigues et al. (2013) suggested the relevance of physical fitness for youth development.

It is important to study the relationships of leptin with aerobic capacity because the latter is connected with fat metabolism and some studies indicate leptin as a possible performance-enhancing hormone in athletes (Pettersen et al. 2001, Kramer et al. 2002) and indicating the efficacy of the combination of exercise and leptin administration in reducing body mass in obese subjects while neither leptin alone or exercise alone seems to be effective (Morris 2008).

It has been proven that in mice leptin deficiency predisposed to worsening neuromechanical upper airway function and administration of leptin increased minute ventilation, suggesting that it's central effect may be attributable to a generalised increase in ventilatory drive. Furthermore, both obesity and leptin deficiency were associated with elevations in passive air resistance in respiratory tract and with marked decrease in active pharyngeal neuromuscular responses (Polotsky et al. 2012).

There are also evidences that the gene expression of calcineurin, CaM-dependent phosphatase, increased in leptin-treated rats (Morishita et al. 1998). Activation of calcineurin in skeletal myocytes selectively up-regulates slow-fiber-specific gene promoters (Chin et al. 1998). This effect could link leptin to increased oxygen consumption. While leptin is being produced mainly by subcutaneous fat tissue, hexogen leptin in deficient subjects may stimulate oxidative phosporylation, mitochondrial biogenesis, and insulin signalling, resulting in improved aerobic exercise capacity and metabolic homeostasis (Miller et al. 2001). Hyperleptinemia in the obese may reflect resistance to leptin at a cellular level and declined aerobic capacity (Frank et al. 2007). This result is confirmed by a study of Leao Da Silva et al. (2012), showing that in obese post pubertal boys the leptin level predicts improvement in static (but not while exercising) lung function after weight loss independently of BMI changes.

There are some studies that can be helpful in understanding the leptin/ VO_{2peak} association because they consider energy expenditure at rest rather than VO_{2peak} in association with leptin levels (Nagy et al. 1997, Salbe et al. 1997, Bishop 1999). In prepubertal children, Nagy et al. (1997) did not find either a

direct effect of leptin or an indirect effect of FM (through leptin) on any measure of energy expenditure at rest. In contrast, Salbe et al. (1997) found in a sample of 123 5-year-old Pima Indian children a significant correlation between rest energy expenditure measured by the doubly labelled water method and leptin levels. These differences can be explained with the inclusion of females in the Salbe et al. (1997) study, known to have higher levels of leptin compared to males. However, the difference in physical activity, body composition and eating habits may influence the relationship between energy expenditure and leptin.

Studies on the relationship between leptin levels and VO_{2peak} in adults show a positive correlation (Ostlund et al. 1996, Miller et al. 2001). Very few studies exist on the relationship between leptin and VO_{2peak} in children and adolescents (Roemmich et al. 1998). In a sample of 16 prepubertal and 13 pubertal normal and overweight boys was not found any relationship. Also, leptin levels are known to increase with puberty (Roemmich et al. 1998). Study the relationship between leptin and VO_{2peak} is not completely clear in children of different BMI, because the existing previous studies have yielded conflicting results, failing to find any associations in normal subjects (Roemmich et al. 1998, Hosick et al. 2010) or were conducted with normal and overweight children but not with obese children (Hosick et al. 2010).

In summary, leptin has a general effect of reducing body weight in obese boys and can contribute indirectly to the improvement of upper airway function, while it can stimulate aerobic capacity and VO_{2peak} improving substrate availability (fat) in trained subjects (Ostlund et al. 1996, Miller et al. 2001).

2.5 Relationships Between Different Parameters of Physical Activity and Leptin

Several studies have investigated the interaction between physical activity and leptin in obese children (Barbeau et al. 2003, Metcalf et al. 2009). Metcalf et al. (2009) did not find in children (mean age 8 years) any associations of objectively assessed physical activity (average physical activity and moderate-to-vigorous physical activity by accelerometry) with leptin concentrations either before or after controlling for body fat. Jimenez-Pavon et al. (2012) indicate in their study on the correlations between physical activity and leptin that vigorous physical activity and fitness moderate the levels of fat mass in late adolescence. Healthy children are recommended to undertake 30 to 60 minutes of moderate-to-vigorous physical activity daily (Pate and O`Neill 2012), consisting of mandatory school physical education classes and extra-curricular organised sport activities. As a rule, different physical activity programs (several weeks or months), decreased body FM and leptin levels (Barbeau et al. 2003). The effect of physical activity on leptin might be modulated by the sympathoadrenal system that has a significant function in ensuring energy balance (Morris 2008).

Several studies have assessed the relationship between physical activity and leptin in non-obese peripubertal boys, but found conflicting results. Martinez-Gomez et al. (2012) showed that vigorous physical activity (as measured by accelerometer) showed a significant inverse correlation with leptin in 13–14-year-olds. A negative (Romon et al. 2004), positive (Salbe et al. 1997), or no significant correlation (Platat et al. 2006, Metcalf et al. 2009, Jimenez-Pavon et al. 2012) with leptin was presented in previous studies in young people, examining their physical activity by different questionnaires. There is no information concerning leptin levels being associated with moderate or vigorous exercises in non-obese 10–12-year-old boys of the same biological maturation stage. This information (especially high leptin, normal BMI) will be useful in better understanding the development of obesity and its metabolic risk in peripubertal boys. There are several recommendations how to measure and distribute children by their physical activity level. Armstrong (2012) recommended to apply the following distribution: leisure-time physical activity, sport-time physical activity, school break-time physical activity and home-time physical activity. However, recently Kristensen et al. (2013) measured the school variation in physical activity, aerobic fitness, and organized sport participation among a large random sample of 9- and 15-year-old Danish children. They concluded that school class has a significant influence on physical activity both in and outside school hours. There is no information about the relationship of leptin with organized out-of-school competitive sport activities in 10–12-year-old boys with normal BMI and Tanner stage two.

2.6 Summary

It emerges from literature that the relationships of leptin with different anthropometric parameters, aerobic capacity and levels of physical activity in normal, overweight and obese peripubertal boys is not completely understood. The role of leptin with the distribution of body fat depots of subcutaneous fat tissue, as energy substrate and its link with physical activity is still unclear. Leptin, as a hormone that is involved mostly in fat metabolism and linked to fat depots, can be associated to subcutaneous fat distribution in the body, but no studies have been conducted on a large sample of peripubertal boys (normal BMI, overweight and obese) investigating this relationship and exploring the possible link between leptin levels and localized body fat depots (e.g. with a specific skinfold thickness or circumference). Leptin can also be linked to energy production by means of the aerobic pathway but very few studies have investigated the relation between energy expenditure and leptin levels and there are no studies exploring the relationships of the VO_{2peak} with leptin in peripubertal boys of different body mass (BMI). The relationship between the physical activity level and leptin has not been investigated in boys with normal BMI in the developmental period (Tanner stage two). However, this relationship is important because, as a rule, physical activity increases aerobic capacity and decreases body fat mass, eventually decreasing the leptin concentration.

3. AIMS AND PURPOSES OF THE DISSERTATION

The aim of this dissertation is to study how leptin correlates with skinfold thicknesses and circumferences, aerobic capacity (peak O_2 consumption) and physical activity parameters (different duration and intensity levels) in peripubertal boys.

The specific aims of the investigation are:

1. To assess possible relationships of leptin with skinfold thicknesses and circumferences of body segments in different BMI subgroups of peripubertal boys (Study I).
2. To evaluate in the same groups of subjects the relationship between leptin levels and peak O_2 consumption (Study II).
3. To examine the relationships between physical activity of different intensity and duration and leptin levels (low, normal, high) in physically active 10–12-year-old boys with normal BMI (Study III).

We hypothesized that:

- Skinfold thicknesses measured in the trunk (abdominal) region have the highest correlations with leptin. There is a high correlation between leptin and the waist-to-hip ratio. Circumferences have a weak correlation with leptin.
- There is a significant correlation in boys with the relative VO_2 peak(ml $\times$ min $^{-1}$ $\times$ kg $^{-1}$) and leptin levels that are not dependent on BMI.
- Leptin has a significant correlation with moderate-to-vigorous physical activity in physically active boys with normal BMI.

4. MATERIAL AND METHODS

4.1 Subjects

The subjects of the Study I and II were from a cross-sectional sample of 248 boys (age 10–12 years) from different schools of the city of Tartu and surroundings (Estonia). The subjects were divided according to Cole et al. (2000) into three BMI subgroups: normal weight, n=190 (BMI<19.8–21.9); overweight, n=34 (BMI<24.0–26.8) and obese, n=24 (BMI≥24.0–26.8). The boys had not been treated for obesity and did not change their eating habits for the time of participation in the above studies. From the total group 94 healthy boys were selected for Study III according to the following criteria: (1) normal BMI by Cole et al. (2000), (2) regular participation in obligatory physical education lessons at school two-to-three times per week, as well as additional participation in organised extra-curricular sport activities for competitive purposes (sport schools, sport clubs, etc.) at least two times per week. The boys were distributed into three subgroups by leptin concentration: normal (X±0.5 SD, 1.2–3.9 ng/ml), low (X–0.5 SD, <1.2 ng/ml) and high (X+0.5 SD, >3.9 ng/ml). The difference between groups was one SD. All subjects in the three studies were mostly at Tanner stage two (ranging from one to four). The boys were not using any medication during the study period and did not have any chronic, genetic or endocrine diseases.

Subjects participating in different studies are summarised in Table 1.

Table 1. Participants in different studies.

	Age (years)	Groups	n
Study I	10 to 12	Total	248 *
		Normal BMI	190
		Overweight	34
		Obese	24
Study II	10 to 12	Total	248*
		Normal BMI	190
		Overweight	34
		Obese	24
Study III	10 to 12	Total	94**
		Normal leptin	44
		Low leptin	31
		High leptin	19

* Different physical activity levels

** Regular participation in organised sport activities outside school at least two times per week. Normal BMI (Cole et al. 2000).

This study was approved by the Medical Ethics Committee of the University of Tartu (Estonia). All subjects and their parents were informed about the purposes and contents of the study. The written informed consent was obtained from the parents before launching the study, and the participants themselves gave a verbal consent.

4.2 Anthropometric Measurements (Study I)

Body height was measured using a Martin metal anthropometer to the nearest 0.1 cm with a standard technique. Body mass was measured with minimal clothing to the nearest 0.05 kg using a medical electronic scale (A&D instruments, Abingdon, UK) and BMI was calculated as body mass (kg) divided by body height squared (m^2).

All skinfold thicknesses and circumferences were measured according to the protocol recommended by the International Association for the Advancement of Kinanthropometry (ISAK) (Marfell-Jones et al. 2006). In total, 9 skinfolds (biceps, subscapular, triceps, iliac crest, supraspinale, abdominal, front thigh, medial calf, and mid axilla) were measured twice with a Holtain (Crymmych, UK) skinfold calliper at the right side of the body with limbs relaxed. If the difference between the 2 measurements was >2 mm, a third measurement was taken and the 2 closest measurements were averaged. Nine skinfold thicknesses were also summarised as an indicator of total body fat.

Thirteen circumferences (head, neck, arm relaxed, arm flexed and tensed, forearm, wrist, chest, waist, hip, mid-thigh, thigh, calf, and ankle) were measured twice using a metal tape from Centurion kit (Rosscraft, Canada). Waist-to-hip ratio was calculated. Both skinfold thicknesses and circumferences were measured by a well-trained anthropometrist (ISAK level 1 anthropometrist).

4.3 Sexual Maturity Assessment (Studies I–III)

Pubertal development was assessed according to the method of Tanner (1962) using self-assessment of genitalia and pubic hair stage in boys. The subjects were given photographs, figures and descriptions and asked to choose from these descriptions the one that most accurately reflected their appearance. In case of discrepancies between the two variables (genitalia and pubic hair stage), greater emphasis for determining the Tanner stage was placed on the degree of genital development. A skilled observer with many years of practical experience attended the assessment in case the subjects had questions.

4.4 Leptin Measurements (Studies I–III)

A 10 ml blood sample was obtained from the antecubital vein with the participant sitting in an upright position in the morning (8–9 a.m.) after an overnight

fast. The serum was separated and frozen at –80°C for later analysis, which was completed within 6 month from collection. Leptin concentrations were determined by an ELISA sandwich method using a kit from Mediagnost GmbH (Reutlingen, Germany). The intra- and inter-assay CVs were less than 10%. The advantage of ELISA sandwich method is that the sample does not have to be purified before analysis, and the assay can be sensitive. Leptin was analysed in the Biochemistry Laboratory of the Institute of Biochemistry of University of Bologna (Italy).

4.5 Peak Oxygen Consumption Measurements (Study II)

Peak O_2 consumption was measured directly using an incremental exercise test protocol until volitional exhaustion on an electronically braked cycle ergometer (Corival V3, Lode, Netherlands). The initial work rate was 60W with increments of 25W after every three minutes until volitional exhaustion. The pedalling rate was set at 70 rpm. The subjects were verbally encouraged to produce the maximal effort. The expired gas during the cycle ergometer test was sampled continuously in breadth-by-breadth mode for the measurement of O_2 consumption using a portable open circuit spirometry system (MetaMax 3B, Cortex, Leipzig, Germany). All data were calculated by means of computer analysis using standard software (MetaMax-Analysis 3.21, Cortex, Leipzig, Germany). Peak O_2 uptake (VO_{2peak}, $l \times min^{-1}$) was measured and VO_{2peak} per kilogram of body mass was calculated. VO_{2peak} consumption values were considered acceptable when two of the following three criteria was met (Petersen et al. 2001):

1. VO_2 plateau defined as a failure of oxygen uptake to increase by more than 2.0 $ml \times kg^{-1} \times min^{-1}$ with increased test load;
2. HR≥95% from the predicted individual maximum (formula 220 – age);
3. Respiratory exchange ratio ≥1.05.

4.6 Physical Activity Measurement (Study III)

The subjects reported their physical activity level using a shortened questionnaire from the Estonian Children Personality, Behavioural and Health Study (Harro et al. 2001) with minor adaptation (Lakka et al. 1994, Kowalski et al. 1997). The modified version of the questionnaire consisted of 8 questions about different physical activity levels (see Table 10) that were directly connected with the aims of the study.

4.7 Statistical Analysis

All statistical analyses were made using the SPSS (version 18.0 for Windows, SPSS Inc. Chicago, USA). Normal distribution of data was controlled and data which were not normally distributed were log transformed. Descriptive statistics (mean±SD) were calculated. Statistical analysis for the skinfolds, circumferences and leptin relationships were differentiated between groups using ANOVA (LSD post-hoc). Partial correlations were used to establish relationships between leptin, skinfolds and circumferences after controlling for age and pubertal status (Study I). Stepwise regression analysis was performed to find out which measured parameter predicts leptin concentration most after controlling for pubertal status and age. Leptin was inserted in the model as a dependent parameter, all skinfolds and circumferences were inserted as independent parameters. Only parameters that correlated significantly with leptin were inserted in the model (Study I).

In the leptin and peak O_2 consumption study (Study II) partial correlations were used to find relationships between leptin concentration and peak O_2 consumption, controlling for age and pubertal status. Stepwise regression analysis was performed to find out which parameter of peak O_2 consumption ($l \times min^{-1}$ or $ml \times min^{-1} \times kg^{-1}$) affects leptin concentration most after controlling for age and pubertal status. Leptin was inserted in the model as a dependent parameter and VO_{2peak} and $VO_{2peak/kg}$ as independent parameters.

In the leptin and physical activity study (Study III), differences between subgroups were analysed using ANOVA (LSD post-hoc). Partial correlations, controlling for BMI and body mass, were used to establish relationships between leptin concentration and the measured physical activity parameters. The level of significance was set at $p<0.05$ for all statistical analyses in all three studies.

5. RESULTS

5.1 Relationship of Anthropometry with Leptin (Study I)

Mean (±SD) anthropometric parameters and leptin are presented in Table 2. No significant differences emerged between the groups in mean age. The subjects in the obese group were taller, compared with the normal group (p<0.05). Obese boys were also taller than overweight boys (p<0.05). Body mass, BMI and leptin differed significantly between all three subgroups. The highest mean leptin concentration was observed in the obese group (Table 2). Mean Tanner stages were similar between the groups (p>0.05, Table 2). However, there were boys at Tanner Stages from one (n=52) to four (n=2).

Table 2. Anthropometric data, leptin and Tanner stages of the subjects (Mean±SD)

Variables	Total (n=248)	Normal (n=190)	Overweight (n=34)	Obese (n=24)
Age (years)	11.18±0.65	11.16±0.65	11.13±0.56	11.36±0.74
Body height (cm)	149.5±7.7	148.5±7.5	151.7±6.4*	155.1±8.4*
Body mass (kg)	43.3±11.9	38.4±6.0	52.6±5.7*	69.4±11.6*#
BMI (kg/m^2)	19.2±4.2	17.3±1.7	22.7±1.5*	29.2±3.2*#
Leptin (ng/ml)	7.63±10.92	3.38±4.10	13.71±8.14*	32.61±13.91*#
Tanner stage (1/2/3/4/5)	2.0±0.6 52/154/40/2/0	1.9±0.7 46/115/27/2/0	2.2±0.6 4/21/9/0/0	2.1±0.5 2/18/4/0/0

*Significantly different from the normal group, p<0.05
#Significantly different from the overweight group, p<0.05

Mean (±SD) skinfold thicknesses and circumferences are presented in Table 3. All variables (skinfold thicknesses, circumferences) were significantly different between the normal, overweight and obese groups in all cases.

Table 3. Mean (±SD) skinfold thicknesses and circumferences in the total group and groups of normal, overweight and obese boys.

Variables	Total (n=248)	Normal (n=190)	Overweight (n=34)	Obese (n=24)
Skinfold thicknesses (mm)				
Triceps	11.6±5.9	9.1±3.2	16.7±4.6*	23.8±4.0*#
Subscapular	8.6±6.4	5.8±2.3	14.2±6.2*	22.0±6.5*#
Biceps	6.4±4.0	4.7±2.1	9.9±3.3*	14.9±2.4*#
Iliac crest	16.1±10.7	11.6±6.4	27.3±8.0*	35.5±7.9*#

Variables	Total (n=248)	Normal (n=190)	Overweight (n=34)	Obese (n=24)
Supraspinale	9.9±8.4	6.2±3.2	17.5±6.4*	27.8±8.5*#
Abdominal	13.0±9.6	8.9±5.1	22.9±7.8*	31.2±7.6*#
Front thigh	17.6±9.7	13.4±5.0	25.9±6.2*	38.9±5.9*#
Medial calf	12.2±6.4	9.7±3.5	16.3±3.9*	25.9±6.7*#
Mid – axilla	8.0±6.5	5.2±2.7	13.4±5.3*	22.3±6.0*#
Sum of 9 skinfolds	103.1±64.1	74.6±29.6	164.2±42.8*	242.3±43.4*#
Circumferences(cm)				
Head	54.2±1.7	53.9±1.6	54.8±1.6*	56.0±1.4*#
Neck	29.4±2.4	28.6±1.7	31.2±1.9*	33.5±2.2*#
Arm flexed	23.8±3.6	22.4±2.1	27.0±2.1*	31.1±2.5*#
Arm relaxed	22.2±3.7	20.7±2.1	25.6±2.0*	29.8±2.6*#
Forearm	21.5±2.0	20.7±1.2	23.1±1.2*	25.5±2.0*#
Wrist	14.2±1.1	13.9±0.8	15.0±0.8*	16.1±1.1*#
Chest	74.2±8.2	70.9±4.6	81.2±5.2*	90.9±7.1*#
Waist	65.1±9.4	61.0±4.4	73.0±5.5*	86.0±7.9*#
Hip	78.2±9.0	74.5±5.0	86.0±4.7*	96.7±7.6*#
Thigh	46.1±7.2	43.0±3.8	52.3±3.6*	61.7±5.3*#
Mid- thigh	42.6±6.0	40.1±3.4	47.7±3.1*	55.2±4.5*#
Calf	30.3±3.6	28.9±2.2	33.1±2.0*	37.7±3.3*#
Ankle	20.7±2.2	19.9±1.5	22.2±1.3*	24.4±2.8*#
Waist- to-hip ratio	0.83±0.05	0.82±0.04	0.85±0.05*	0.89±0.73*#

* Significantly different from the normal group, $p<0.05$
# Significantly different from the overweight group, $p<0.05$

From the anthropometric parameters, body height was related to leptin only in the total group, but not in the three subgroups, controlling for puberal status and age (Table 4). Body mass correlated significantly with leptin only in the total and normal BMI groups and, finally, leptin did not correlate significantly with the BMI in the obese group (Table 4). In the total group, the relationship between separate skinfold thicknesses and leptin was higher than r=0.70 (except for medial calf, Table 4). In subgroups of total, normal BMI and overweight boys, the relationships between all skinfold thicknesses and leptin were significant (Table 4). In the obese group, the relationships with triceps, biceps and front thigh skinfolds were not significant ($p>0.05$, Table 4). The sum of 9 skinfold thicknesses correlated significantly with leptin in all groups (r=0.558–0.779, Table 4).

Table 4. Partial correlations between leptin, skinfolds and circumferences in boys after controlling for pubertal status and age.

Variables	Total (n=248)	Normal (n=190)	Overweight (n=34)	Obese (n=24)
Body height	0.210***	0.084	–0.221	–0.019
Body mass	0.663***	0.305***	0.161	0.294
BMI	0.715***	0.402***	0.386*	0.285
Skinfold thicknesses				
Triceps	0.741***	0.523***	0.768***	0.332
Subscapular	0.745***	0.487***	0.521**	0.433*
Biceps	0.741***	0.486***	0.562***	0.191
Iliac crest	0.768***	0.531***	0.592***	0.444*
Supraspinale	0.747***	0.468***	0.595***	0.524*
Abdominal	0.746***	0.516***	0.697***	0.505*
Front thigh	0.740***	0.506***	0.682***	0.123
Medial calf	0.652***	0.387***	0.480**	0.689***
Mid – axilla	0.744***	0.460***	0.738***	0.451*
Sum of 9 skinfolds	0.779***	0.558***	0.755***	0.561**
Circumferences				
Head	0.328***	0.124	0.259	0.103
Neck	0.486***	0.031	0.466**	0.232
Arm flexed	0.683***	0.333***	0.642***	0.187
Arm relaxed	0.694***	0.363***	0.676***	0.246
Forearm	0.606***	0.239***	0.325	0.099
Wrist	0.449***	0.016	0.250	0.041
Chest	0.640***	0.238**	0.469**	0.401
Waist	0.702***	0.373***	0.481**	0.344
Hip	0.696***	0.395***	0.489**	0.353
Thigh	0.724***	0.444***	0.564***	0.334
Mid – thigh	0.691***	0.382***	0.423*	0.366
Calf	0.609***	0.231**	0.160	0.326
Ankle	0.495***	0.107	0.384*	0.265
Waist–to-hip ratio	0.333***	0.020	0.190	0.038

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

In the total group, all measured circumferences correlated significantly ($p<0.001$) with leptin (Table 4; $r=0.328–0.724$) controlling for pubertal status and age. In the normal BMI group, leptin relationship with head, neck, wrist and ankle circumference was not significant. In the overweight group head, forearm, wrist and calf circumferences did not correlate significantly with leptin (Table 4). Surprisingly, in the obese group none of the measured circumferences correlated significantly with leptin (Table 4). Waist-to-hip ratio correlated significantly with leptin only in the total group (Table 4).

Stepwise regression analysis results are presented in Table 5. All skinfold thicknesses and circumferences were used in separate groups. In the total group,

the sum of 9 skinfolds was the main predictor of leptin concentration ($R^2 \times 100 = 30.7\%$). From the circumferences, thigh and wrist were the most important ($R^2x100 = 28.9\%$). In the normal BMI group, the sum of 9 skinfolds together with supraspinale skinfold thickness characterized leptin concentration best ($R^2 \times 100=33.1\%$). From the circumferences, the most important were thigh and calf ($R^2 \times 100 = 23.9\%$). In the overweight group, the best predictor of leptin was triceps from skinfold thicknesses ($R^2x100=66.4\%$) and relaxed arm circumference ($R^2 \times 100 = 55.6\%$). In the obese group, the medial calf skinfold thickness characterized leptin concentration by 49.0%. No significant relationships with the measured circumferences and leptin emerged in the obese group (Table 5).

Table 5. Stepwise regression analysis between leptin and skinfold thicknesses and between leptin and circumferences after adjusting for pubertal status and age.

Model	R^2x100	β	p
Total (n=248)			
Sum of 9 skinfolds	30.7	0.545	<0.001
Thigh circumference	28.9	0.663	<0.001
Wrist circumference		–0.390	<0.001
Normal (n= 190)			
Sum of 9 skinfolds	33.1	0.813	<0.001
Supraspinale skinfold		–0.283	0.067
Thigh circumference	23.9	0.693	<0.001
Calf circumference		–0.317	0.004
Overweight (n= 34)			
Triceps skinfold	66.4	0.697	<0.001
Arm relaxed circumference	55.6	0.655	<0.001
Obese (n= 24)			
Medial calf skinfold	49.0	0.688	<0.001
Circumferences	–	–	–

5.2 Relationship of Leptin with Peak Oxygen Consumption (Study II)

Mean (±SD) anthropometric parameters, Tanner stages, leptin and peak O_2 consumption in different groups are presented in Table 6. There were no significant ($p>0.05$) differences between groups in mean age and Tanner stage. The subjects in the obese group were the tallest (Table 6). Peak O_2 consumption was significantly higher ($l \times min^{-1}$) or significantly lower ($ml \times min^{-1} \times kg^{-1}$) in the overweight and obese groups, respectively (Table 6). Leptin was significantly higher ($p<0.05$) in the overweight and obese groups compared to the normal BMI group. All the subjects were mostly at Tanner stage two (Table 6).

Table 6. Mean (±SD) anthropometric parameters, Tanner stage, peak O_2 consumption and leptin concentration in boys.

Variables	Total group (n=248)	Normal group (n=190)	Overweight (n=34)	Obese (n=24)
Age (years)	11.18±0.65	11.16±0.65	11.13±0.56	11.36±0.74
Body height (cm)	149.5±7.7	148.5±7.5	151.7±6.4*	155.1±8.4*
Body mass (kg)	43.3±11.9	38.4±6.0	52.6±5.7*	69.4±11.6*#
BMI ($kg \times m^2$)	19.2±4.2	17.3±1.7	22.7±1.5*	29.2±3.2*#
Leptin (ng/ml)	7.63±10.92	3.38±4.10	13.71±8.14*	32.61±13.91*#
VO_{2peak} ($l \times min^{-1}$)	1.96±0.33	1.91±0.32	2.08±0.29*	2.21±0.31*
$VO_{2peak/kg}$ ($ml \times kg^{-1} \times min^{-1}$)	47.02±8.34	50.05±6.20	40.50±5.72*	32.38±4.30*#
Tanner stage (1/2/3/4/5)	2.0±0.6 52/154/40/2/0	1.9±0.7 46/115/27/2/0	2.2±0.6 4/21/9/0/0	2.1±0.5 2/18/4/0/0

* Significantly different from the normal group, $p<0.05$.
# Significantly different from the overweight group, $p<0.05$

Partial correlations between leptin and VO_{2peak} where age and Tanner stage were eliminated are presented in Table 7. Interestingly, the absolute peak O_2 consumption ($l \times min^{-1}$) correlated significantly with leptin only in the total group. In different BMI subgroups the relationship was not significant ($p>0.05$). Contrary, relative VO_{2peak} ($ml \times min^{-1} \times kg^{-1}$) highly correlated with leptin. The negative relationship was the highest in the total group (Table 7, r=–0.674). In all subgroups this relationship was significant as well ($p<0.05$).

Table 7. Partial correlations (controlling for age and Tanner stage) between leptin and peak O_2 consumption in boys.

Variables	Total group (n=248)	Normal group (n=190)	Overweight (n=34)	Obese (n=24)
VO_{2peak} ($l \times min^{-1}$)	0.196**	–0.017	–0.155	–0.144
$VO_{2peak/kg}$ ($ml \times kg^{-1} \times min^{-1}$)	–0.674***	–0.247***	–0.464**	–0.468*

* $p<0.05$
**$p<0.01$
***$p<0.001$

In Table 8 are presented the results of the regression analysis. The relationship between leptin and VO_{2peak} (both $l \times min^{-1}$ and $ml \times min^{-1} \times kg^{-1}$) was very high only in the total group ($R^2 \times 100=53\%$) where the group consists of boys with different BMI. In the normal body mass, overweight and obese groups only VO_{2peak} has significant relationship with leptin (7.3%, 35.8% and 24.2%, respectively, $R^2 \times 100$).

Table 8. Regression analysis with VO_{2peak} ($l \times min^{-1}$) and $VO_{2peak/kg}$ ($ml \times kg^{-1} \times min^{-1}$) as independent variables and leptin as the dependent variable after controlling for age and Tanner stage.

Model	$R^2 \times 100$	β	p
Total group (n= 248)			
VO_{2peak} ($l \times min^{-1}$)	53.0	0.314	<0.001
VO_{2peak} ($ml \times kg^{-1} \times min^{-1}$)		–0.705	<0.001
Normal group (n= 190)			
VO_{2peak} ($ml \times kg^{-1} \times min^{-1}$)	7.3	–0.247	0.001
Overweight (n= 34)			
VO_{2peak} ($ml \times kg^{-1} \times min^{-1}$)	35.8	–0.422	0.007
Obese (n= 24)			
VO_{2peak} ($ml \times kg^{-1} \times min^{-1}$)	24.2	–0.487	0.028

5.3 Relationship of Leptin with Physical Activity (Study III)

Mean anthropometric parameters and leptin characteristics are presented in Table 9. The mean age differed nonsignificantly between groups. Subjects in the high leptin group were the tallest ($p<0.05$). Body mass, BMI and leptin differed significantly in all three subgroups.

Table 9. Anthropometric parameters and leptin in boys. Mean ± SD.

Variables	Total group (n=94)	Low leptin group (n=31)	Normal leptin group (n=44)	High leptin group (n=19)
Age (yrs)	11.2±0.7	11.0±0.7	11.3±0.7	11.1±0.6
Body height (m)	1.47±0.71	1.46±0.82	1.48±0.63	1.50±0.80$^{\#}$
Body mass (kg)	37.5±5.3	34.9±4.7	37.4±4.5*	42.1±5.4$^{*\#}$
BMI (kg/m^2)	17.2±1.5	16.4±1.1	17.1±1.3*	18.7±1.5$^{*\#}$
Leptin (ng/ml)	3.08±2.76	0.86±0.43	2.75±0.81*	7.47±2.89$^{*\#}$

* Significantly different from low leptin group, $p<0.05$.
$^{\#}$Significantly different from normal leptin group, $p<0.05$.

Physical activity parameters are presented in Table 10. No significant differences emerged between subgroups. There were no significant relationships between leptin and the measured physical activity parameters, except between leptin and moderate physical activity performed at least five times per week (each session at least 30 min, r=–0.608) in the high leptin group.

Table 10. Physical activity parameters in boys (X±SD).

Activity parameters	Total (n=94)	Low leptin (n=31)	Normal leptin (n=44)	High leptin (n=19)
For how many days were you physically active last week, at least 30 min per day (0–7)	4.0±1.4	4.2±1.4	3.9±1.5	3.9±1.2
Participation in PE lessons in school last academic year (1–5)	4.5±0.5	4.5±0.5	4.4±0.5	4.6±0.5
For how many months in the last year did you take part in sport sessions outside school – in sport schools, sport clubs, etc. (0–12)	8.8±2.9	8.1±3.1	8.9±2.9	9.4±2.7
How many times per week did you take part in organised sport sessions (0–7)	3.0±1.0	3.0±0.9	3.1±1.2	3.1±0.9
What was the duration of one organised sport session (min)	78.5±22.3	76.0±17.7	77.5±20.9	85.0±30.7
How frequently were you active outside school near your home playing with friends, etc. (times per week) (0–4)	1.9±0.7	1.8±0.7	1.9±0.8	1.9±0.6
Last week, was your moderate physical activity equal to or higher than a 30 min session 5 times per week (1-yes, 2–no)	1.4±0.5 Yes – 56 No – 38	1.4±0.5 Yes – 19 No – 12	1.4±0.5 Yes – 25 No – 19	1.3±0.5 Yes – 12 No – 7
Last week, was your vigorous-to-moderate physical activity equal to or higher than a 30 min session 3 times per week (1–yes, 2–no)	1.1±0.3 Yes – 78 No – 16	1.1±0.3 Yes – 27 No – 4	1.2±0.4 Yes – 36 No – 8	1.4±0.3 Yes – 15 No – 4

6. DISCUSSION

6.1 Relationship of Anthropometric Parameters with Leptin (Study I)

The results of this study indicate that in all BMI subgroups the sum of 9 skinfolds related highly with leptin in peripubertal boys. From the circumferences, the most important were thigh circumferences (together with wrist) in total and normal BMI (together with calf) groups. In the overweight group only the relaxed arm circumference was selecte and in the obese group there were no significant relationships between circumferences and leptin concentration. It is interesting that in the overweight and obese subgroups the measurement points of the parameters selected for the model were only on limbs. Our results were surprising, because leptin concentration is dependent first of all on the sum of 9 skinfolds which is connected with the total subcutaneous fat tissue mass.

Several researchers have found strong positive relationships for leptin with BMI, % body fat and especially fat mass in children and adolescents (Ellis and Nicloson 1997, Rutters et al. 2008). Leptin is mainly produced in white adipose tissue and circulating as the content of adipose tissue (Considine et al. 1996). However, there is few information available about the influence of subcutaneous fat distribution to leptin concentration in children and adolescents with different BMI. In our study, all 9 measured skinfold thicknesses correlated significantly with leptin in the total, normal BMI and overweight groups (Table 4). This finding is in accordance with earlier published research according to which leptin is mainly produced by subcutaneous white fat tissue (Zhang et al. 2005), and adipocyte size is an important determinant of leptin secretion (Skurk et al. 2007).

In the total and normal BMI groups the most important predictor of leptin concentration was the sum of 9 skinfolds (Table 5). This is in accordance with other studies, indicating that the fat distribution is not important but the total fat mass is (Ellis and Nicloson 1997, Kelli et al. 2012). It is difficult to explain the relatively low (but significant) relationships between leptin and different skinfold thicknesses in the obese group (Table 4) in our study. Using stepwise multiple regression analysis only medial calf was selected to correlate significantly with leptin (Table 5). In the research where the subjects were of the same age and covered the same BMI groups as the obese boys in our study, Sudi et al. (2001) concluded that subcutaneous adipose tissue layers measured with Lipometer as not compressed fat tissue, significantly correlated with leptin in obese boys. In contrast, in lean peripubertal boys a weak correlation was found between adiposity measures and leptin (Sharrock et al. 2008). In our study, in the total, normal BMI and overweight groups all measured skinfold thicknesses correlated significantly with leptin. However, in the obese group the triceps, biceps and front thigh skinfolds did not correlate significantly with leptin (Table 4). There is evidence that leptin levels in prepubertal boys increase during the beginning of pubertal development and then remain stable in spite of the

increasing BMI (Mantzoros et al. 1997). However, Roemmich and Rogol (1999) concluded that leptin concentrations reflect the size of the subcutaneous fat depot better than total fat mass or abdominal visceral fat in boys. Secondly, a peak of leptin concentration between 11 and 13 years has been shown at 11 years in boys (Rutters et al. 2008). Changes of puberty are observable in boys concerning the ratio of fat free mass being fat mass changes faster during puberty (Rico et al. 1993, Mantzoros et al. 1997, Kiess et al. 1999, Gueorguiev et al. 2001). However, there is evidence that the rate of leptin production is related to adiposity, but a large portion of the interindividual variability in leptin concentration is independent from body fatness (Jequier 2002).

From the circumferences, the most frequently used one for predicting coronary artery disease risk factors are waist and hip circumferences and their ratio. In our study the waist and hip circumferences correlated separately and significantly with leptin in all groups, except the obese group (Table 4). In previous studies the waist and hip circumferences have not correlated with leptin after adjustment for fat mass in obese children (Sudi et al. 2001). The waist-to-hip ratio did not correlate significantly with leptin in 13-year-old boys (Kettaneh et al. 2007). Only in the study of Mann et al. (2003) of obese boys, the leptin levels associated with waist-to-hip ratio in a positive manner. The waist circumference correlated significantly with leptin ratio in Pima Indian children (Misra et al. 2001, Yamborisut et al. 2009). However, the waist circumference is more related to visceral fat than to total body fat (McCarthy 2006).

Different measurement errors can occur in using skinfold callipers or tapes for the measurement of subcutaneous adipose tissue thicknesses and circumferences, especially in the overweight and obese groups, so this method has to be compared with results yielded by DXA. Secondly, we did not take into account the food intake in our subjects. The strength of our complex study was that for the first time the detailed skinfold thicknesses (9 skinfolds) and especially 13 circumferences were used for the analyses conducted on the same subjects. At present no complex studies have been documented in literature where a great number of skinfold thicknesses and circumferences are compared as parameters of body fat distribution in boys. To our knowledge, this study is the first to present evidence for the relationships between leptin and separate skinfold thicknesses and circumferences in peripubertal boys. Our study indicates that further studies are necessary investigating the relationships between leptin, fat mass development and metabolic consequences and considering that subcutaneous fat mass is measured with maximum accuracy. From the two indirect measurement methods applied, the sum of 9 skinfolds is more acceptable for identifying the relationship with leptin compared to the measurement of circumferences in different groups of pubertal boys.

Our study indicates that leptin concentration is highly dependent on the skinfold thicknesses and circumferences in total and normal BMI groups of peripubertal boys whereas in the overweight and obese subgroups this relationship is not so strong. Some measured parameters in limbs are not sensitive to

leptin concentration. In the obese the circumferences did not correlate significantly with leptin. The main finding of the current study was that all measured skinfold thicknesses and circumferences correlated highly with leptin in the total group. However, in the normal BMI group, the relationship of measured circumferences (head, neck, wrist and ankle – all with very low fat mass) with leptin was not significant. Thus, it is acceptable to consider skinfold thicknesses and circumferences only in the normal BMI group. Especially in very obese boys better methods are needed for measuring body fat, because BMI can indicate "overweight" rather than "over fatness". Close relationships emerged in our study between leptin and all measured skinfold thicknesses (9) and circumferences (13) in boys. However, in obese boys the measured circumferences were not significant parameters for leptin concentration. When comparing the total and normal BMI groups of boys, the most important feature connecting leptin and body fat, is the sum of 9 skinfolds, characterizing the total body topography of skinfold thicknesses. The lack of relationship between specific body skinfolds and leptin in obese is partially attributable to the higher occurrence of errors in measuring skinfolds at high levels of adiposity and its lower reproducibility in children (Ulijaszek and Kerr 1999).

6.2 Peak Oxygen Consumption and Leptin (Study II)

Sedentary lifestyle together with elevated body fatness is considered to be a significant predictor of cardiovascular disease in youth (Andersen et al. 2006). Peak oxygen consumption is considered to be the main parameter of aerobic fitness (Saavedra et al. 2011) and directly connected with health (cardiovascular disease risk). Decrease in body mass can significantly increase $VO_{2\ peak/kg}$ (Goran et al. 2000). On the other hand, the intensity of exercise seems to be a key factor in training design (Wenger and Bell 1986). Aerobic fitness has proved to be significantly associated with adiposity in childhood (Rodrigues et al. 2013), but there are very few data confirming this finding, especially in peripubertal children (McNarry and Jones 2013). Our results indicate that leptin is significantly related to relative VO_{2peak} ($ml \times min^{-1} \times kg^{-1}$) in all groups. The negative relationships in the obese groups were stronger than in the normal BMI group. However, the relationships were strongest in the total group (53.0%, $R^2 \times 100$) (Table 7). The relationship with peak O_2 consumption ($l \times min^{-1}$) was insignificant, except in the total group.

The mean values of $VO_{2peak}/_{kg}$ were relatively high in the subjects of the current study. For example, in our total group the mean value was higher than recommended cut–offs (in 8–11-year-old boys 43.6 $ml \times min^{-1} \times kg^{-1}$) by Adegboye (2011) and 47.02±8.34 $ml \times min^{-1} \times kg^{-1}$in our study (Table 6). Our mean results are higher in the obese group compared with the slightly higher BMI (32.9±4.8 $kg \times m^2$) measured in obese children by Andreacci et al. (2005). There is evidence that the difference between the absolute cardiovascular fitness of normal-weight and overweight children need not always reach statistical

significance (McGavock et al. 2009). On the other hand, cardiorespiratory fitness is regarded as an important marker of boys' health due to its association with obesity (Ortega 2008). One reason for a relatively high $VO_{2peak/kg}$ is that our subjects were healthy boys, some of them participating in sport clubs.

The mean leptin levels in our study differed greatly between groups (Table 7), this is typical for other studies as well (Roemmich and Rogol 1999). There are very few studies about the relationships between leptin and energy expenditure. In animal models the injection of leptin in mice resulted in increased O_2 consumption (Pelleymounter et al. 1995). Contrary, the data obtained by Nagy et al. (1997) do not support the hypothesis that leptin concentration (independent of fat mass) is related to measured energy expenditure in children (Nagy et al. 1997). Finally, the high rates of fat utilization decline in boys during maturation (Stephens et al. 2006).

Very few studies have investigated the connection of leptin and peak O_2 consumption. In our study highly significant correlations emerged between leptin and $VO_{2peak/kg}$ in all measured groups (Table 7). Only one study by Roemmich et al. (1998) confirmed these results in prepubescent boys and girls. Stene-Johannessen et al. (2013) studied a large group of Norwegian nine-year-old children and concluded that directly measured peak O_2 consumption associated independently with leptin levels.

Two recent studies have indicated that indirectly measured VO_{2peak} (20 m shuttle-run test) associated with lower concentrations of leptin in adolescence (Jimenes-Pavon et al. 2012, Martinez-Gomez et al. 2012). One explanation is that lean boys with low fat mass had a low leptin concentration. On the other hand, lean boys also had a relatively high $VO_{2peak/kg}$ (Jimenez-Pavon et al. 2012). The significant correlation of VO_{2peak} ($l \times min^{-1}$) with leptin can be explained with leptin increasing the total energy expenditure.

One of the limitations of our study is that we did not measure the participants' physical activity levels and their dietary intake in a detailed way. More information could be yielded from research results in case the participants were distributed to subgroups on the basis of different leptin values, not solely the differences in BMI. It can be concluded that leptin correlated negatively with the relative peak O_2 consumption and the absolute VO_{2peak} correlated with leptin only in the total group.

6.3 Physical Activity and Leptin (Study III)

The relationships between leptin concentration and organised outside-school sport activity have not been studied before. The main finding of this study was that no significant relationships emerged between leptin level and physical activity in a very selected group (normal BMI, Tanner stage two and regular participation in competitive physical activity practiced at least two times per week) of 10–12-year-old boys. However, in the high leptin subgroup the most

important variable connected with leptin is regular moderate exercise performed at least five times per week, each session lasting for at least 30 min (Table 10).

The measurement of physical activity in youth is a complex area, as evienced by the constantly changing and increasingly sophisticated instruments for measuring it. Our results do not confirm the hypothesis that there is significant correlation between leptin, moderate-to-vigorous physical activity and normal BMI. It has been established previously that only vigorous physical activity correlates with leptin in 13–17-year-old adolescents (Martinez-Gomez et al. 2012) or a mixed group (obese and with normal body mass) of 8–11-year-old girls (Belcher et al. 2013). We presumed that participation in organised sport was not the exercise of sufficiently vigorous intensity. However, the boys in our study represented different sport events, such as basketball, volleyball, soccer, etc. On the other hand, the subjects were too young to specialise to only one sport event and the training mostly consisted of different sport games, elementary sport event-specific exercises, studying sport technique, etc. Playing sport games in physical education lessons at school can also represent vigorous physical activity. However, Dudley et al. (2012) have recently concluded in their research of a large group of secondary school students that the percentage of class time spent in moderate to vigorous physical activity was only 56.9% of the total time devoted to physical activity. This is probably not sufficient for causing significant relations between leptin and physical activity. The respective guidelines elaborated in the US state that adolescents should accumulate 60 minutes of moderate-to-vigorous physical activity per day (Physical Activity Guidelines for Americans, 2008). However, a recent study by Leek et al. (2011) indicates that less than one-fourth of youth athletes reached the recommended 60 minutes of moderate-to-vigorous physical activity level during their sport practices. Finally, time spent in moderate-to-vigorous physical activity correlated negatively with changes in BMI from age 9 to 15 (Mitchell et al. 2013). One explanation of the above effect is that high-intensity physical activity *per se* is a metabolic regulator for the sensitivity of several hormones that can consequently affect leptin concentration (Jimenes-Pavon et al. 2012). On the other hand, moderate-intensity physical activity results in reduced abdominal fat for leptin synthesis (Racette et al. 1997). Recently, Campos et al. (2013) concluded that aerobic plus resistance training decreases leptin concentration in adolescent boys. In our study the regular physical activity could have decreased the body fat mass at least in some boys. However, leptin remained at high levels for reasons unknown. We can hypothesize that the cessation of regular exercise for boys with high leptin will result in increased body mass that in its turn increases the leptin concentration. Simply, increasing the number of boys who not do regularly aerobic exercises, increased leptin concentration (r=–0.608)

It is well known that there are large variations in leptin concentration among individuals with similar BMI through childhood and adolescence (Clayton et al. 1997). There is evidence that leptin concentration does not always mirror the body mass loss in children (Cambuli et al. 2008). The same holds true in our study, as we distributed the boys into three subgroups using one SD for the

criterion. It was interesting that the only significant correlation between leptin and physical activity of at least five times per week for at least 30 min was found in the high leptin group. This high leptin group consisted only of boys who practised sport games, were tall and with higher BMI than the boys representing other sport events (Table 9). Finally, Park et al. (2012) concluded that high initial leptin levels could predict greater BMI and metabolic risk score in the future. In the same study they concluded that the influence of leptin on body mass and BMI seems to be independent from children's physical activity.

During puberty, leptin concentration reaches a peak at Tanner stage two for boys (Roemmich et al. 1998, Romon et al. 2004). This can explain why especially in high leptin group the mean concentration of leptin was very high in absolute terms. In the low leptin group, especially in slim boys, the proximity threshold could have hampered the detection of the effects of physical activity on leptin level(Romon et al. 2004).

Some limitations in our study deserve additional mentioning. First, the cross-sectional design of the study. Second, the subjects' physical activity level was assessed only on the basis of self-reported (questionnaire-based) data. Third, our results are valid only for the selected group of 10–12-year-old boys who are physically active, have normal BMI and are at Tanner stage two. Fourth, we did not take into account the influence of behavioural and demographic variables of the subjects and the boys were from one region (Tartu and its surroundings).

We studied the correlations between physical activity in non-obese healthy peripubertal boys with different leptin concentrations. If the leptin concentration is high in boys with normal BMI then vigorous physical activity is not necessary, for lowering the leptin level it is enough to exercise systematically with moderate intensity. The novelty of this study is that we have established significant correlations between physical activity of moderate intensity and leptin level in boys belonging to the high leptin subgroup. The boys with normal BMI whose leptin is elevated should know the respective reasons, avoid the risk of being overweight and exercise regularly at moderate intensity.

It can be concluded that the correlations between physical activity and leptin are weak in peripubertal boys of normal body mass. Only regular moderate physical activity correlated significantly with leptin in the high leptin subgroup. Relatively intensive sport participation outside school and taking part in obligatory physical education lessons is not a significant factor for influencing leptin concentration.

6.4 Anthropometry, Aerobic Capacity and Physical Activity Responses to Leptin in Peripubertal Boys

Children's health is first of all dependent on the anthropometric parameters that characterize the amount of body fat depots (skinfold thicknesses and circumferences) since subcutaneons adipose tissue represents about 85% of all body fat mass. The major metabolic role of adipose tissue is regulating the storage and

metabolization of lipid energy. Both aerobic fitness (peak O_2 consumption) and physical activity are correlated with anthropometric parameters, first of all those characterizing body fat.

Leptin has been shown to be the most important signalling hormone linking body fat depots (substrate availability) and energy expenditure. The relationships of leptin with subcutaneous fat distribution topography that can have different lipolytic activity levels, is not well studied, especially the influence of different circumferences. Our study that is conducted on a large number of peripubertal boys and comprises the measurement of 9 skinfold thicknesses and 13 circumferences, using the method recommended by ISAK (Marfell-Jones et al. 2006) confirms that the all measured skinfold thicknesses correlate significantly with leptin in the total, normal BMI, overweight and obese subgroups. Stepwise regression analysis confirms that in the total and normal BMI groups the sum of 9 skinfolds (characterizing indirectly total body fat mass) is the best parameter characterizing leptin concentration. In overweight and obese subgroups the skinfold thicknesses of limbs proved to be the most important ones in the above context. Better methods are probably needed for the measurement of body fat because skinfold callipers are not valid for measuring skinfold thicknesses in overweight and obese boys. The current study represents an original approach to complex relationships between body circumferences and leptin. In the obese group of subjects no link could be established between the measured circumferences and leptin and the probable explanation here could be the differences either in skeleton size (somatotype) or in the amount of muscle tissue. Consequently, circumferences are not the best parameters that characterize body fat mass in the obese and for this reason no significant correlations with leptin emerged.

For decreasing body fat mass several programs of physical exercises are recommended combined with nutrition control. Exercising increases energy expenditure and decreases body fat mass that is likely to decrease the influence of blood leptin concentration. In our study we used a simple questionnaire for measuring physical activity of different intensity and duration. The positive effect of training programs containing aerobic exercises on decreasing body fat mass in obese children is well known. However, in the subjects of our study – physically active boys with normal BMI – the leptin concentration was quite different. Than first time we studied subgroups of boys with normal, high or low leptin. It is interesting that in these selected groups the correlation between leptin and physical activity was weak, significant correlation emerged only between leptin and regular moderate aerobic physical activity in high leptin subgroup. In this subgroup the boys' body height and body mass were slightly higher than in other subgroups. Previous studies have emphasized the health-related importance of physical activity with the minimum of moderate-to-vigorous intensity. There is possibly a link between aerobic exercises (which decrease body fat mass), and leptin concentration in relatively well-trained peripubertal boys with normal BMI. It is necessary to emphasize that the

participants in our study were highly selected because belonging to the some community and practising sports.

Exercising as a rule decreases both body mass and body fat mass. High aerobic capacity (mostly via regular exercising) directly decreases several metabolic syndrome risk factors, including body fat mass. Relative (per kg body mass) peak O_2 consumption is highly dependent on both body mass and body fat mass. Still now there were not available any studies in a large sample of peripubertal boys about that correlations between peak O_2 consumption (both in absolute or relative units) and leptin. In our study we found the negative significant correlation between leptin and relative peak O_2 consumption ($ml \times min^{-1} \times kg^{-1}$). In boys with different anthropometric and body energy consumption parameters the correlation between leptin and peak O_2 consumption was significant only in the total group. We have shown the negative correlation of leptin from VO_2 peak/kg in all other subgroups (boys with normal BMI, overweight and obese boys). Then different body fat mass and different leptin groups (normal BMI, overweight, obese) had a negative correlation with VO_2 peak/kg. Systematic aerobic exercising with relatively high energy expenditure probably increases VO_2 peak/kg that decreases body fat mass and leptin concentration in blood.

Leptin has been shown to be the most important signalling hormone linking physical activity, body fat tissue mass and aerobic capacity. To study peripubertal boys at a similar peak growth velocity are less studied. We presented new but partly conflicting results about the correlations between leptin, anthropometry, peak O_2 consumption and physical activity of different intensity level. However, there were some methodological limitations in our study – measurement body fat mass and distribution (weak significant correlations with different circumferences, even with nonsignificant correlations with well known waist-to-hip ratio in selected normal BMI, overweight or obese groups). The second main limitation is the assessment of physical activity using only a simple modified questionnaire instead of e.g. ActiGraphs. In the future it will be interesting to know leptin-physical activity relationships in the longitudinal perspective during the whole puberty period to understand the reasons why the leptin level in boys with similar BMI and physical activity level differs to a great extent.

7. CONCLUSIONS

In conclusion, our results are:

1. We found a significant correlation of leptin concentration with all skinfold thicknesses and the sum of 9 skinfold thicknesses. In the total group all measured circumferences related significantly to leptin. In the obese group none of the measured circumferences correlated with leptin levels.
2. Relative ($ml \times min^{-1} \times kg^{-1}$) peak aerobic capacity correlated negatively with leptin levels in peripubertal boys in all groups: total, normal BMI, overweight and obese. The obese subjects showed the strongest negative association between leptin levels and absolute peak aerobic capacity ($l \times min^{-1}$).
3. No significant correlations emerged between leptin and physical activity levels of different duration and intensity in peripubertal boys with normal BMI. In the high leptin group regular moderate physical activity correlated significantly with leptin.

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9. SUMMARY IN ESTONIAN

Multifunktsionaalse hormooni leptiini seos antropomeetriliste näitajatega, aeroobse töövõimega ning kehalise aktiivsusega puberteedi alguses olevatel poistel

SISSEJUHATUS

Varasemad uuringud on näidanud, et rasvumisega on otseselt seotud mitmed adipotsütokiinid. Üheks tähtsamaks markeriks tuleks siin lugeda leptiini, mida sünteesitakse põhiliselt valges rasvkoes. Leptiin on organismis seotud eelkõige söögiisu regulaatorina ning energiavahetuse tasakaaluga. Siiski individuaalsed erinevused vere leptiini kontsentratsioonis on suhteliselt suured samavanustel poistel, kes on ühesuguse kehalise aktiivsusega ja keha rasva kaaluga. Kindlalt on tõestatud leptiini seos kehamassi indeksiga (KMI) ning keha rasvkoe kaaluga.

Üksikutes uuringutes on uuritud nahaaluse rasvkoe jaotuvuse seoseid leptiiniga. Ka uuringuid kehalise aktiivsuse mõjust leptiini kontsentratsioonile leidub suhteliselt vähe. Kehalise aktiivsuse uuringutes omab suurt tähtsust treeningkordade arv nädalas, ühe treeningtunni kestus ning eriti treeningute intensiivsus. Sageli just viimane tegur on otsustava tähtsusega. Kõige suuremat tähtsust omab siiski keha energeetiline tasakaal. Kui energeetilised kulutused on suuremad kui toiduga saadav energia siis reeglina rasvkoe kaal hakkab vähenema ja sellega väheneb ka leptiini kontsentratsioon veres. Siin omab tähtsust ka maksimaalse pingutuse aegne organismi hapnikutarbimise võime, mis reeglina sportides põhjustab suurema energijakulu ja seoses sellega ka väiksema rasvkoe kaalu. Nendel teemadel on uuringuid läbi viidud eelkõige ülekaalulistel täiskasvanutel. Oluliselt vähem on uuritud poisse puberteedi alguses. Seoses eelpool öelduga, viidi läbi kompleksuuring, kus püüti välja selgitada leptiini seoseid nii keha eelkõige rasvkoe kaalu mõjutavate antropomeetriliste näitudega (nahavoltide paksused ja ümbermõõdud), aeroobse võimekusega, kui ka kehalise aktiivsusega.

Uurimistöös püstitati järgmised hüpoteesid:

1. Nahavoltide paksused, mõõdetuna kehatüve piirkonnas on usutavas seoses vere leptiini sisaldusega. Ka talje ja puusa ümbermõõtude suhe on otseselt seotud leptiiniga. Siiski erinevate ümbermõõtude seos leptiiniga on mitteusutav.
2. Suhteline maksimaalne hapniku tarbimine ($ml \times min^{-1} \times kg^{-1}$) on otseses seoses vere leptiini sisaldusega.
3. Seos vere leptiini sisalduse ja mõõduka ning intensiivse kehalise aktiivsusega on usutav.

Uurimistöö hüpoteeside lahendamiseks püstitati järgmised ülesanded:

1. Võrrelda seoseid vere leptiinisisalduse ja nahavoltide paksuste (9) ning ümbermõõtude (13) vahel erineva KMI-ga puberteedieelistel poistel, kes bioloogiliselt vanuselt kuuluvad keskmiselt Tanner 2 gruppi (uuring 1).

2. Leida seoseid leptiinisisalduse ning individuaalse hapniku tarbimise võime vahel erineva KMIga poistel (uuring 2).
3. Leida seoseid erineva kehalise aktiivsuse (intensiivsus, treeningtunni kestus jne) ja vere leptiinisisalduse vahel normaalse KMI–ga poistel.

UURITAVAD JA METOODIKA

Uuritavateks esimeses ja teises uuringus olid 248 10 kuni 12 aastast poissi, kes vastavalt KMIle (Cole jt 2000) jaotati kolme gruppi: normaalne KMI (n=190), ülekaalulised (n=34) ja rasvunud (n=24). Eelpool esitatud gruppidesse kuulusid väga erineva kehalise aktiivsusega poisid. Samuti kasutati koguvalimit (n=248).

Kolmandasse uuringusse selekteeriti kehaliselt aktiivsed poisid (vähemalt 2 korda nädalas süstemaatilist harjutamist spordikoolis/ringis). Nende kõigi KMI oli normi piires ning bioloogiliselt arengult kuulusid Tanner 2-e. Selles grupis vastavalt individuaalsele leptiinisisaldusele jaotati poisid kolme gruppi- keskmine (n=44), madal (n=31) ja kõrge (n=19). Gruppidevaheliseks erinevuseks võeti üks standardhälve.

Nahavoltide paksused ja ümbermõõdud mõõdeti Rahvusvahelise Kinantropomeetriaühingu (Marfell-Jones et al. 2006) soovituste järgi. Hapnikutarbimist määrati tõusvatel koormustel veloergomeetril suutlikkuseni. Hapniku tarbimist määrati otseselt kasutades gaasianalüsaatorit.

Kehalist aktiivsust määrati küsimustiku abil. Esitati kokku 8 küsimust kehalise aktiivsuse kordade arvu kohta nädalas, ühe treeningtunni kestuse ning intensiivsuse kohta. Küsiti ka osalemist kooli kohustuslikes kehalises kasvatuse tundides.

Kõikides uuringutes määrati vere leptiinisisaldust firma Mediagnost (Saksamaa) komplekti abil.

JÄRELDUSED

1. Tervikgrupis (n=248) kõik mõõdetud nahavoltide paksused korreleerusid usutavalt leptiiniga. Väga tugev on ka seos 9 nahavoltide summaga. Tervikgrupis kõik ümbermõõdud olid usutavas seoses leptiiniga. Rasvunute grupis need usutavad seosed puudusid täielikult, $p > 0.05$.
2. Leptiin on negatiivselt seotud suhtelise hapniku tarbimisega ($ml \times min^{-1} \times kg^{-1}$). Absoluutne hapniku tarbimine ($l \times min^{-1}$) on leptiiniga seotud ainult tervikgrupis.
3. Reeglina leptiin ei korreleeru usutavalt kehalise aktiivsuse näitajatega poistel. Ainult regulaarne mõõdukas kehaline aktiivsus korreleerub usutavalt leptiiniga kõrge leptiinisisaldusega grupis.

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